

ANTIMICROBIAL ACTIVITY OF LACTOBACILLUS STRAINS AGAINST ANTIBIOTIC-RESISTANT PATHOGENS IN VAGINAL INFECTIONS

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ABSTRACT

In today's environment of increasing resistance to antibiotics, it is very important to identify the types of resistant bacteria and use agents with antimicrobial activity against them.

By studying the activity of vaginal lactobacilli against vaginal pathogens, the probiotic properties of lactobacilli in preventing cervical pathogens from causing disease were studied.

Keywords: lactobacillus, bacterial vaginitis, *S.aureus*, antibiotic resistance.

INTRODUCTION

The diverse bacterial flora in the female genital tract significantly influences vaginal health and overall flora balance. Vaginal infections are among the most prevalent female health issues, often leading to severe complications and contributing to the high incidence of gynecological disorders. Bacterial vaginosis (BV) is the most frequent cause of abnormal vaginal discharge in women of reproductive age [1, 2]. The composition of microbial flora in the vagina and cervix varies across different stages of health, development, and disease.

Lactobacillus bacteria, which are typically found in the vaginal microflora, play an essential role. A reduction in lactobacilli numbers in patients suffering from various conditions, such as urinary tract infections, bacterial vaginosis, and other genital infections—including cervical cancer—can lead to significant health issues, warranting careful attention [3,4]. Generally, lactobacilli are not linked to disease and are regarded as non-pathogenic members of the intestinal and vaginal flora [5]. They help sustain a healthy vaginal environment by inhibiting the growth of pathogenic bacteria.

Regulatory processes Lactobacillus species produce antibacterial compounds such as lactic and other organic acids, hydrogen peroxide (H₂O₂), and bacteriocins.

Bacteriocins are biologically active, low molecular-weight proteins or peptides that inhibit the growth of a variety of pathogenic bacteria which appear in the bacterial vaginosis or cervicovaginal infections.

MATERIALS AND METHODS

Isolation of bacterial vaginal infection pathogens (BV).

Vaginal pathogens were isolated from vaginal cultures of women with clinical BV at Aisha (AIWA) medical center (Tashkent) and Karima hospital (Tashkent), whose diagnosis was confirmed by significant BV. Appropriate vaginal fluid samples were cultured on MHA (himedia) soft agar (tryptic soy broth with 0.7% agar, BBL, Microbiology System, Md) under aerobic conditions at 37°C for 18-24 hours, and pH values were determined.

Isolation of bacteriocinogenic strains of lactic acid bacteria from various sources

The substrate for isolation of LAB (fermented vegetables - medicinal plants and women swabs sample) placed in MRS broth and cultured at 37 ° C for 48 hours for enrichment. Serial dilutions were prepared from the enriched broth and sown as a continuous lawn on MRS agar, 2 dishes for each dilution. 1 dish from 2 replicates was placed in a thermostat under aerobic cultivation conditions, the second - in an anaerobic jar, where the air was replaced with gaseous nitrogen (under anaerobic conditions). Cultivated at 37 ° C for 48 hours. After cultivation, isolated colonies differing in morphological features were selected from the dishes, then transferred to a dish with MRS agar containing 2-3 drops of an alcohol solution of the bromocresol purple indicator to determine acid formation. The dishes were incubated at 37°C until growth appeared. Isolates that changed the color of the medium from purple to yellow were preliminarily considered to be LAB and used for further studies.

Bacterial identification and susceptibility testing

The identification of bacteria isolated from urine samples were performed by Maldi-TOF. Initially, the urine samples are cultured on Mueller Hinton agar (Himedia) and were incubated at temperature 35–37°C.

Study of the sensitivity of isolates to antibiotics

Sensitivity to antibiotics was studied using the method of Da Cunha L R. et al. [5]. The sensitivity of all 5 uropathogens isolates to 10 antibiotics was studied – representatives of different groups, most frequently used in clinical practice – roxithromycin (30 µg), chloramphenicol (30 µg), Tobramycin (10 µg), penicillin-G (10 unit), cefazolin (30 µg), tetracycline (30 µg), Clarithromycin (15 µg), cefoperazone/Sulbactam (30 µg) (HiMedia, India). The diameter of the growth-free zone was measured and the ratio of each culture to antibiotics was assessed as resistant (R), moderately resistant (SR) and sensitive (S) according to the supplier's instructions.

The analysis was performed in triplicate. The sensitivity of isolates of pathogens causing dermatological infections was studied using the same method using disks with antibiotics. In addition to the antibiotics listed above, the following disks with antibiotic were used: oxacillin (1 µg), (HiMedia, India).

Study of antimicrobial and bacteriocinogenic activity of isolates

Antagonistic properties of isolates against pathogens in vitro were analyzed by the spot method described by Da Cunha L.R. et al. [6]. For this purpose, lactobacilli grown in MRS broth (HiMedia, India) for 24 hours were centrifuged at 7000 x g for 20 minutes at 4°C. After removing the bacterial cells, the cell-free supernatant was heated in a water bath at 90°C for 15 minutes to inactivate proteases and prevent potential contamination. Then, stationary phase indicator bacteria at a concentration of 10⁶ CFU/ml were spread on Mueller-Hinton agar plates. After drying the plates for 5 minutes, 5 µl of the isolated supernatant was spotted onto the BV pathogenic bacteria. After overnight incubation at 37°C, the inhibition zone in the indicator layer was observed.

RESULTS

Lactobacillus isolation

The vaginal samples from 50 healthy and diseased women were diluted and cultured on MRS agar plates. Colonies (80) were mostly obtained from the surface of MRS agar. Most of them showed round, small colonies without pigment and white to cream in color. They were all tested later

Gram staining, catalase production and spore formation are examined. Only 5 of them showed characteristics of gram-positive, catalase-negative and non-sporulating lactobacilli. Finally, 5 potent antimicrobial isolates were selected for further analysis.

Samples taken from 100 patients with vaginal infections yielded both Gram-positive and Gram-negative bacteria. A total of 150 bacterial isolates were identified using MALDI-TOF (table-1). Six bacterial pathogens were selected using selective media, and pure cultures were maintained on nutrient agar for further investigation. The cultures included *Candida albicans*, *E. coli*, *E. faecalis*, *S. epidermidis*, and *K. pneumoniae*.

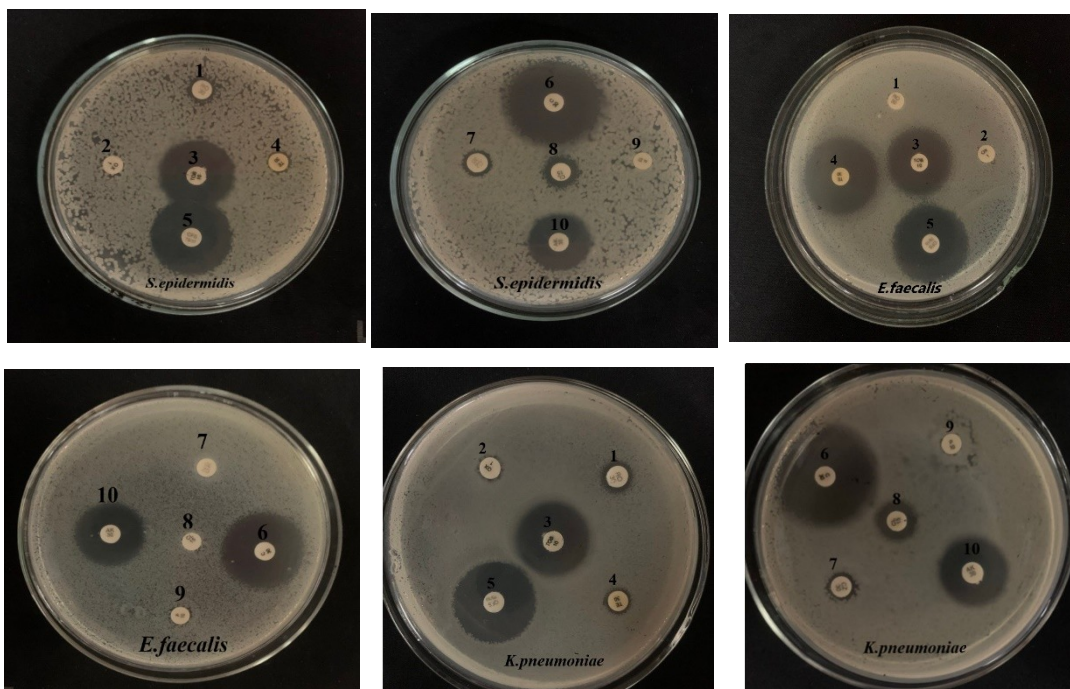
Table- 1 *Bacteria isolated from vaginal infection*

Microorganism	Overall %
Gram positive bacteria	
<i>S. epidermidis</i>	6%
<i>Staphylococcus pasteurii</i>	3%
<i>Staphylococcus aureus</i>	8%
<i>Enterococcus faecalis</i>	2%
<i>Enterococcus faecium</i>	2%
<i>Lactobacillus concavus</i>	0.1%
<i>L.paracasei</i>	0.1%
Gram negative bacteria	
<i>E.coli</i>	23%
<i>Klebsiella pneumoniae</i>	2%
<i>Proteus mirabilis</i>	5%
<i>P. aeruginosa</i>	2%
<i>Pseudartrobacter olychromoges</i>	1,6%
<i>Psrevibacillus invocatus</i>	1,6%
Fungi	
<i>Candida albicans</i>	8.3%
<i>Candida tropicalis</i>	3.3%
<i>Candida galabrata</i>	3.3%
<i>Candida krusei</i>	1.6%
Total	100%

The resistance of selected bacteria to various groups of antibiotics was studied. It was found that the *E. coli* strain did not exhibit resistance to any antibiotic group. *K. pneumoniae* showed resistance to β -lactam antibiotics and tetracycline. The *Staphylococcus epidermidis* strain exhibited resistance to penicillin G, cefazolin, and tetracycline antibiotics. Among the bacteria studied, *E. faecalis* demonstrated the highest resistance, showing resistance to macrolides, β -lactam antibiotics, and cefazolin.

№	Antibiotics		Growth inhibition zone diameter, mm				
		Class of antib.	Conc. µg/disc	<i>E.coli</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus epidermidis</i>	<i>Enterococcus faecalis</i>
1	Roxithromycin	I	30	10	5	30	R
2	Clarithromycin		15	8	10	25	R
3	Oxacillin	II	10	25	R	4	R
4	Penicillin-G		10	48	R	R	R
5	Tobramycin	III	10	32	24	10	22
6	Amikacin		30	30	20	27	20
7	Tetracycline	IV	30	40	R	R	28
8	Cefoperazone/Sulbactam	V	30	31	28	20	30
9	Chloramphenicol	VI	5	14	30	5	25
10	Cefazolin	VII	30	46	5	R	R

Note: I. Macrolides; II. β -lactam; III. Aminoglycoside; IV Tetracycline; V. sulfonamides. VI. Chloramphenicol; VII. cephalosporin

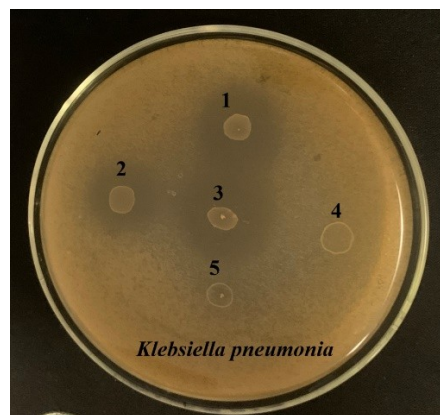
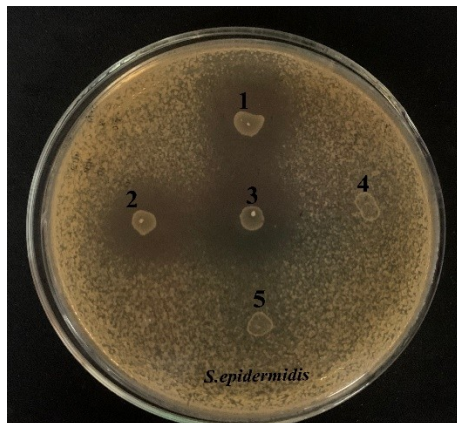


Note: 1. Roxithromycin 2. Oxacillin 3. Tobramycin 4. Tetracycline 5. Cefoperazone/ Sulbactam 6. Chloramphenicol 7. Cefazolin 8. Clarithromycin 9. Penicillin 10. Amikacin

Antibiotic resistance is a global health concern that has shifted attention towards alternative treatments. Probiotic therapy, which involves the oral or intravaginal administration of Lactobacilli to restore healthy microbial balance, has shown promise [7]. Antimicrobial activity of lactobacilli isolated from patients against pathogenic bacteria was studied. The results showed that the activity of 3 bacteria isolated from plant sources is stronger than lactobacilli isolated from vaginal microflora.

Table 1 Comparison of average inhibitory zones of six lactobacillus strains against four representative BV causing tract infection

Lactobacillus strains		Lactobacillus strains Diameter of Inhibitory zone (mm)				
		<i>C. albicans</i>	<i>E.coli</i>	<i>E.faecalis</i>	<i>S.epidermidis</i>	<i>K.pneumoniae</i>
1	<i>L.plantarum-1</i>	34	18	13	16	24
2	<i>L.plantarum-2</i>	28	15	18	15	12
3	<i>L.plantarum-3</i>	28	21	28	21	20
4	<i>L.delbrueckii</i>	0	0	0	0	0
5	<i>L.paracasei</i>	0	0	0	0	0



Of the five Lactobacillus strains obtained in the study, three were isolated from plants, and the remaining two were isolated from the vaginal flora. The smallest activity of *L. plantarum* strains was observed against the *K. pneumoniae* strain, with an inhibition zone of 12 mm. The highest activity was recorded against *C. albicans*, with a 34 mm inhibition zone. Additionally, the lactobacilli isolated from

vaginal microflora did not exhibit antimicrobial activity against pathogenic bacteria isolated from the vagina.

Conclusion

Bacterial vaginosis (BV), a polymicrobial vaginal infection in reproductive-age women, is a significant health concern. BV often disrupts the balance between vaginal microflora and opportunistic bacteria, potentially leading to gynecological complications such as premature birth, miscarriage, and endometritis. The use of lactobacilli offers a promising approach to combat antibiotic resistance and restore the natural balance of the vaginal microbiome

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